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Immobilisation alters the heat shock response of *Salmonella* Typhimurium

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Summary

Our work shows, that the heat shock response of planktonic *Salmonella* is NOT the same, as the heat shock response of immobilised *Salmonella* when it comes to flagella and virulence gene regulation.

We show that this difference in heat shock response of immobilised *Salmonella* is not restricted to a transcriptomic level, but also results in a different phenotype in motility and invasion assays.

This study implies that data obtained from heat shock studies of planktonic *Salmonella* can not readily be translated to *Salmonella* grown immobilised.

Introduction:

Research into stress mechanisms of *Salmonella* have often focused on growth in liquid broth cultures (planktonic growth) or *Salmonella* engaged in biofilm formation. Immobilised growth is an often overlooked form of growth encountered in e.g. nature or contaminated food.

To investigate to which extent immobilisation affect the heat shock response of *Salmonella* Typhimurium, we compared the heat shock response of immobilised *Salmonella* to that of planktonic controls.

Experimental outline:

Salmonella Typhimurium 4/74 was grown in the GelCassette system developed at the Institute of Food Research, Norwich, UK. The cassettes were filled with either broth (planktonic growth) or broth supplemented with a gelling agent (immobilised growth).

The cassettes were incubated at 25° C until 10^8 CFU/ml (planktonic) or 10^8 CFU/g (immobilised). The bacteria were isolated and RNA extracted for transcriptomic analysis.
A second set of cassettes were grown as described above followed by incubation at 45° C for 30 minutes. After the heat shock, bacteria were isolated and RNA extracted for transcriptomic analysis.

Flagella

Heat shock of planktonic *Salmonella* leads to a down regulation of many flagella genes.

Heat shock of immobilised *Salmonella* leads to an up regulation of many flagella genes.

Bacterial flagella are filamentous organelles that drive cell locomotion. To investigate if the changes in flagella gene regulation results in altered motility we performed a swimming assay (figure 1).

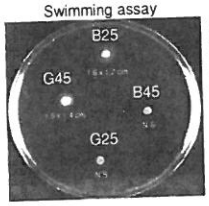

Figure 1: Salmonella Typhimurium 4/74 were grown and heat treated as stated in experimental outline before isolation from the cassettes and spotting on motility plates. Plates were incubated at 37° C for 5.5 hours.
B25: Planktonic Salmonella
B45: Heat shocked planktonic Salmonella
G25: Immobilised Salmonella
G45: Heat shocked immobilised Salmonella
NS= No swimming zone

Heat shock of planktonic *Salmonella* leads to a decrease in motility.

Immobilisation in it self leads to a decrease in motility, which can be restored by the heat shock.

These data indicate that the transcriptomic differences in heat shock response between planktonic and immobilised *Salmonella* translates into different phenotypes.

Virulence

Salmonella has several virulence genes some of which are important for invasion of host cells. Without invasion of host cells *Salmonella* will not be able to cause systemic infection.

Heat shock of planktonic *Salmonella* leads to down regulation of several virulence genes.

Heat shock of immobilised *Salmonella* leads to an up regulation of several virulence genes.

To investigate if the heat shock induced changes in virulence gene expression, has an impact on invasion, an infection assay was performed (figure 2).

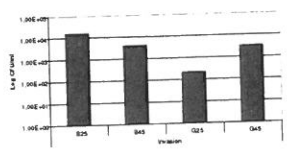

Figure 2: Salmonella Typhimurium 4/74 invasion of HeLa epithelial cells. Salmonella was grown as in experimental outline. MOI 10:1 Salmonella to HeLa cell ratio.
B25: Planktonic Salmonella; B45: Heat shocked planktonic Salmonella; G25: Immobilised Salmonella; G45: Heat shocked immobilised Salmonella

Heat shock of planktonic *Salmonella* leads to a slight decrease in the ability to invade HeLa cells.

Heat shock of immobilised *Salmonella* leads to an increased ability to invade Hela cells.

These data indicate that the transcriptomic differences in heat shock response between planktonic and immobilised *Salmonella* translates into different phenotypes.